

## ON THE ROLE OF THE INDUCER IN THE SYNTHESIS OF MALTASE IN YEAST<sup>1</sup>

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One aspect of the process of the induced synthesis of enzymes which is relatively amenable to experimental analysis is the role of the inducer. It has been established in a variety of cases that utilizability is not a necessary attribute of effective inducers. Thus, Spiegelman *et al.* (1947) demonstrated that maltose can induce maltase at pH values that preclude metabolism of the maltose by fully adapted yeast cells. Analogues of natural substrates have been employed for similar purposes. Thus,  $\alpha$ -methyl glucoside can be shown to be an inducer of maltase at concentration levels at which utilization cannot be detected (Spiegelman, 1948). Monod *et al.* (1951) have demonstrated similarly that melibiose, though not metabolizable by *Escherichia coli*, is nevertheless an extremely powerful inducer of  $\beta$ -galactosidase.

Such experiments eliminated interpretations of inducer function which demand active and detectable metabolic transformations of the inductor, such as might be required were induction to involve the accumulation of unique intermediates derived from the inducer, in the sense suggested by Leibowitz and Hestrin (1945). The data do not exclude mechanisms which involve the conversion or fixation of a very small number of inducer molecules.

More pertinent to the purposes of the present paper, however, is the fact that these experiments leave open the question of whether inducers must form complexes, with the enzyme being formed as a *sine qua non* of induced synthesis of enzyme.

Much of the earlier and even some of the more recent (Mandelstam, 1952; Mandelstam and Yudkin, 1952) speculations on the role of the inducer have assumed such complex formation

as a necessary concomitant of enzyme formation. Yudkin (1938) was the first to provide an explicit formulation of this concept, and he did so in terms of a mass action hypothesis in which a substrate by combining with enzyme drives the reaction toward the conversion of precursor into enzyme.

To explain the kinetics of enzyme synthesis which can be observed under certain conditions, and certain features of the transmission of enzyme forming capacity, Spiegelman (1945, 1946) was led to abandon the simple mass action theory and to suggest a modification which assumed that an autocatalytic element (the plasmagene) was involved in the synthetic process. Subsequently, Monod (1947) proposed an analogous hypothesis with however more restricted genetic autonomy than was inherent in the plasmagene theory. Both the Monod and the Spiegelman modifications, nevertheless, retained the central thesis that the inducer functioned by complexing with the enzyme. The retention of this concept was conditioned not only by its attractive simplicity, but also because it suggests an explicit explanation for the specificity which appeared to characterize the induction of enzyme synthesis. It possessed the added advantage of providing a concrete, and in principle, testable mechanism of at least one reasonably well defined portion of the inductive process.

An uncomplicated application of the complexing concept leads to several predictions, the experimental violation of any one of which would require either its revision or complete abandonment. We may list some of these predictions as follows: (1) Substances which can "complex" with enzyme should be effective inducers. (2) Substances shown to be incapable of complex formation with a given enzyme likewise should be unable to induce this enzyme. (3) The dissociation constant of a substance measured in terms of its inductive effect on enzyme synthesis should be comparable in magnitude to the value obtained in experiments in which the constant is

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derived from the complexant properties of the inducer with enzyme.

It should be noted that the term "complex formation" employed here is meant to subsume both the specific type (characterized by combination between an enzyme and its substrate or a competitive inhibitor) and the nonspecific type (characterized by a complex between an enzyme and a noncompetitive inhibitor). Thus, proof that an inducer does not form a specific "complex" with enzyme does not eliminate it as an enzyme complexant since it leaves open the possibility of nonspecific complex formation. There is no *a priori* reason for believing that combinations of the latter type cannot function in the process of enzyme synthesis. However, violations of prediction (3) above would eliminate the involvement of either complexant type.

The design of experiments to test the validity of these predictions is subject to one important restriction. Thus far, the induction of enzyme synthesis has been achieved only with intact cells. The comparison of the behavior of a substance as an inducer and as a complexant with enzyme must, therefore, be made on the intact cell. Thus, if a substance were shown to be a complexant with the enzyme in an *in vitro* test and exhibited no inductive capacity, it could be argued that it cannot "penetrate" the intact cell. Analogous or comparable arguments can be leveled at successful violations of any of the three predictions which are achieved by comparing information obtained from *in vitro* experiments on enzyme with *in vivo* inductions. The data to be discussed therefore will be confined for the most part to *in vivo* comparisons.

A clear-cut experimental contradiction of the complexing concept came from Lederberg's (1951) examination of mutational effects on the inductive process. He exhibited a mutant of *E. coli* (Lac<sup>-</sup>) which could produce  $\beta$ -galactosidase providing alkyl galactosides were employed as inducers, but which did not respond to lactose despite the fact that the latter is a substrate of the enzyme once formed. This result is in clear contradiction to the first prediction listed.

Monod *et al.* (1951) were the first to subject these questions to a systematic analysis employing the  $\beta$ -galactosidase system of *E. coli*. They reported violations of the first two predictions noted above. Thus, phenyl- $\beta$ -thiogalactoside

was found to be a potent competitive inhibitor, both *in vivo* and *in vitro* of the  $\beta$ -galactosidase of *E. coli*. Despite this, it exhibited no inductive capacity and in addition actually showed itself to be an effective inhibitor of enzyme synthesis in the presence of such inducers as melibiose and methyl- $\beta$ -galactoside.

Interesting information stems from the remarkable results obtained by Pollock (1950, 1952) in his study of penicillinase production by *Bacillus cereus*. Here the combination between the inducer and its cellular co-complexant is virtually irreversible. As a consequence of this relative irreversibility, even fleeting exposures of cells to penicillin at 0 C° can fix a sufficient number of inducer molecules as to enable the cells to form enzyme subsequently when placed in an environment lacking free inducer. An estimation by Pollock (1952) of the dissociation constant of penicillin as an inducer yields a value of  $3 \times 10^{-7}$  M. Pollock (*personal communication*) has in unpublished experiments evaluated the dissociation constant between penicillin and extracellular penicillinase and found a value of  $6 \times 10^{-5}$  M. This 200-fold discrepancy between the dissociation constants of penicillin as an inducer and as a substrate of the enzyme would represent a direct contradiction of prediction (3), had they both been obtained from intact cell measurements. The possibility of a change in  $K$ , value on isolation of an enzyme makes conclusions drawn from such comparisons to that extent uncertain. Such modifications on isolation have in fact been realized in the case of  $\beta$ -galactosidase (Lederberg, 1950) and malic oxidase (Huennekens, 1951).

It is the purpose of the present paper to present experiments relevant to the second and third predictions listed. The system employed is the induction of maltase in yeast by  $\alpha$ -methyl glucoside (AMG) under conditions in which the latter is unutilizable as a substrate. The data obtained indicate that the dissociation constant of  $\alpha$ -methyl glucoside as an *in vivo* enzyme complexant is markedly different from the value obtained when it is employed as an inducer. The data further demonstrate that the low capacity of  $\alpha$ -methyl glucoside to inhibit maltose utilization by the induced system is noncompetitive in nature.

## MATERIALS AND METHODS

*Yeast strain.* The yeast strain used in the present investigation is strain K, a diploid representative of *Saccharomyces*.

*Conditions of culture.* Cells were grown in a complete medium prepared by adding the following to one liter of water: bacto-peptone, 5 g; yeast extract, 2.5 g; sodium lactate (60 %) 6.0 ml; calcium chloride, 0.25 g;  $\text{MgSO}_4$ , 0.25g;  $\text{KH}_2\text{PO}_4$ , 2 g;  $(\text{NH}_4)_2\text{SO}_4$ , 6 g; and glucose, 40 g.

Cultures were incubated in the above medium in standing cotton plugged 125 ml Erlenmeyer flasks, containing 50 ml of medium. Unless otherwise specified all incubations were carried out at 30 C, and exponential phase cells were employed.

*Conditions of induction.* In order to obtain rapid and effective enzyme synthesis, employing  $\alpha$ -methyl glucoside as an inducer, it was found desirable to have the cells suspended in a synthetic medium during the induction. Burkholder's (1934) medium was modified by removing the asparagin and adding 5.9 g of succinic acid to increase the buffering capacity. Then the medium was brought to pH 4.5 by the addition of KOH. Henceforth this medium will be referred to as medium B.

Inductions were conducted in medium B with the addition of various concentrations of glucose and  $\alpha$ -methyl glucoside. They were carried out in large test tubes placed in a water bath at 30 C with stirring accomplished by bubbling either air or nitrogen, depending upon whether aerobic or anaerobic conditions were desired. In all cases the gas was saturated previously with water vapor.

*Stabilization of enzyme content.* To obviate the difficulty of enzyme synthesis in the course of assay for enzyme content, use was made of the discovery by Swenson and Giese (1950) that ultraviolet irradiation stops enzyme formation. Four ml aliquots were irradiated in open petri dishes (7.1 cm in diameter) 15 cm from a low pressure, 15 watt General Electric germicidal bulb. Unless otherwise stated the radiations were carried out for 160 seconds. Suspensions were stirred during irradiation by means of a magnetic stirrer. Following exposure the samples were transferred quantitatively with cold water to a centrifuge tube, harvested, rewashed with cold water, and suspended in 4 ml of cold medium B.

The samples were stored then at 3 C for subsequent assay of enzyme activity. In preliminary experiments no detectable loss of enzyme activity occurred during storage. The dose of ultraviolet given to prevent further enzyme formation reduced the viable count to less than 0.003 per cent and was of a level such that no photo-reactivation of the enzyme synthesizing system was observable on exposure to visible light.

*Preparation of suspension.* Cells were harvested by centrifugation from the growth medium immediately prior to the experiment. They were washed twice in cold water and resuspended in medium B to the desired density with the aid of a Klett-Summerson colorimeter previously calibrated for this purpose. The density employed was such as to contain 2.6 mg dry weight of yeast per ml of suspension.

*Manometric measurements.* Enzyme activity was estimated manometrically employing 3 per cent maltose as substrate. All such measurements were carried out at 30 C with a standard Warburg apparatus under anaerobic conditions. The enzyme activities are expressed in terms of  $Q_{\text{CO}_2}^{\text{N}}$ . For reasons which are not as yet clear, complete expression of maltase content in  $\alpha$ -methyl glucoside induced, ultraviolet stabilized cells is not achieved unless small amounts of glucose are added. It was customary to include one mg of glucose with the substrate. The level of maltase activity was estimated over a 50 to 60 minute period after the glucose added was accounted for as  $\text{CO}_2$ . The  $Q_{\text{CO}_2}^{\text{N}}$  values obtained from intact cells are in excellent agreement with direct measurements of maltase activity in dried cell preparations (Halyorson and Spiegelman, 1952).

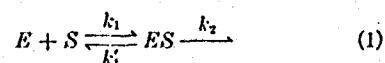
Growth during the induction was checked by both direct and viable counts. Except for one aerobic experiment of over 3 hour duration, no detectable increase in cell number occurred during the period of the experiment. The reason for this stems from two facts. One is that exponential phase cells harvested from the complete medium and inoculated into the synthetic medium B have a lag of 2.5 hours. The other is that the densities routinely employed in the induction correspond to  $2.5 \times 10^8$  cells per ml which is close to the stationary phase level of the organism.

## RESULTS

*Combining constant of  $\alpha$ -methyl glucoside as an inducer of maltase.* A study of the kinetics of

induction with  $\alpha$ -methyl glucoside has been made and is described elsewhere (Spiegelman and Halvorson, 1954, unpublished data). The results obtained reveal that if the energy supply is not restricted, the synthesis of maltase is exponential with time. This makes the determination of the  $K_s$  value of the inducer somewhat more complicated than that encountered in the usual enzyme-substrate experiments. It is of some interest, therefore, to detail the reasoning employed in making the calculations.

The usual derivation of the Michaelis-Menten relation starts with the following reaction sequence:



Here  $E$ ,  $S$ , and  $P$  designate enzyme, substrate, and product respectively, and the  $k$ 's represent the corresponding reaction velocities. Two assumptions are generally made. One is that the velocity of the reaction is proportional to  $[ES]$ , the concentration of the enzyme-substrate complex. The other is that  $k_2$  is considerably smaller than  $k'_1$ . The relation derived from the equilibrium condition enables one to determine the dissociation constant,  $K_s$ , of the  $ES$  complex.

To calculate the  $K_s$  value of inducer as a stimulator of enzyme synthesis, we may start with a similar reaction sequence:



Where  $i$  is inducer and  $\pi$  the cellular component with which it combines. We need not specify the nature of  $\pi$  now except to note that combination between it and inducer is a necessary prelude to significant enzyme synthesis in inducible systems.

We assume, as in the case of the Michaelis-Menten derivation, that the reaction constant  $k_2$ , governing the irreversible destruction of the complex, is small as compared with the back reaction. It is assumed further that the rate of enzyme formation is proportional to the concentration of this complex,  $[\pi i]$ , so that

$$\frac{dE}{dt} = K'[\pi i] \quad (3)$$

Where  $K'$  is constant. The work of Pollock (1952) and of Pollock and Torriani (1953) provides support for both of these assumptions.

From the equilibrium condition of reaction

(2), one can solve for the concentration of the complex. Substituting in equation (3), we have

$$\frac{dE}{dt} = \frac{V[i]}{[i] + K_s} \quad (4)$$

where  $V$  is the rate of enzyme synthesis at inducer saturation for fixed  $[\pi]$  and is equal to  $K'[\pi]$ .  $K_s$  is the dissociation constant of the  $\pi i$  complex. Relation (4) is formally identical to the Michaelis-Menten equation. It cannot, however, be employed as such since in our experimental system  $\frac{dE}{dt}$  is not constant with time. Since the inducer employed is not metabolized,  $[i]$  is constant in any given experiment. Consequently,  $V$  and, therefore,  $[\pi]$  must be functions of time. The nature of the function to be specified is suggested by the observation that the kinetics of enzyme synthesis in yeast under the experimental conditions employed here possess an exponential phase. We assume, therefore, that  $\pi$  varies exponentially with time and increases in the form of the  $\pi i$  complex. The latter takes account of the fact that increase in enzyme-forming capacity does not take place unless inducer is present. We find then that

$$\pi = \pi_0 e^{(V'[i]/([i] + K_s)t)} \quad (5)$$

Where  $V'$  is the "growth constant" of  $\pi$  at saturating inducer concentrations,  $\pi_0$  the value of  $\pi$  at time zero, and  $K_s$  is the dissociation constant of the  $\pi i$ -complex.

Substituting (5) in (4), integrating and evaluating the resulting constant yields in

$$E = K(e^{(V'[i]/([i] + K_s)t)} - 1) \quad (6)$$

From equation (7) it is evident that as soon as  $\frac{V'[i]}{[i] + K_s} t$  becomes significantly greater than unity,  $E$  is approximated by the product of  $K$  and the exponential term. Under these conditions we may write

$$\ln E = \ln K + \frac{V'[i]}{[i] + K_s} t \quad (7)$$

Equation (7) can in principle be used to estimate  $K_s$  from the slopes at various inducer concentrations of the linear portions obtained by plotting  $\ln E$  against time. The use of equation (7), is of course, restricted to the linear parts of the semilogarithmic plots. The relation between the slopes thus determined and the concentrations

of the inducer is, from equation (7), formally identical to the Michaelis-Menten relationship. The usual methods may be employed, therefore, on the resulting data to determine the  $K_s$  value of  $\alpha$ -methyl glucoside as an inducer. Should  $\pi$  turn out to be the enzyme itself, the  $K_s$  so determined should coincide with that obtained in experiments measuring combination between  $\alpha$ -methyl glucoside and the enzyme.

We may summarize here, briefly, the details of how such experiments were carried out. Exponential phase cells were harvested and washed immediately prior to their use. Then they were suspended in medium B to a density of 2.84 mg dry weight of cells per ml. Inductions were conducted at a series of  $\alpha$ -methyl glucoside concentrations in aerated tubes placed in a water bath held at 30 C. An energy source was provided in the form of glucose at a concentration of 3.3 mg per ml. It had been shown in previous experiments (Spiegelman and Halvorson, 1954) that this level does not interfere with the onset or subsequent progress of maltase synthesis. Following the addition of the inducer, samples were removed at intervals, and their enzyme content stabilized with ultraviolet light as described under Methods. The cells then were washed with chilled water, resuspended in 0.1 M phosphate-succinate buffer at pH 4.5, and their enzyme activity assayed manometrically over a period of 60 minutes. At each  $\alpha$ -methyl glucoside concentration tested a minimum of three samples was taken in the exponential period of enzyme formation for the determination of rate of enzyme synthesis. It was found that at the low levels of

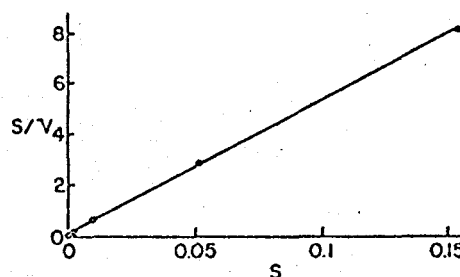


Figure 1. A Lineweaver-Burk plot of  $(S)/v$  against  $(S)$  where  $(S)$  is the molar concentration of  $\alpha$ -methyl glucoside and  $v$  is the rate constant of maltase synthesis. The data employed are those of an experiment detailed in table 1.

glucose employed the cells possessed some constitutive capacity for enzyme formation. Consequently, rates on inductor-free controls were always determined so that the values obtained in the presence of inducer could be corrected to those characteristic of external induction.

Table 1 summarizes a typical set of results obtained in an aerobic induction, and figure 1 exhibits a Lineweaver-Burk (1934) plot of the data. From the latter it is evident that the response of the rate constant of enzyme formation to inducer concentration is described by the Michaelis-Menten relation. The  $K_s$  value may be determined by the usual statistical procedures involving the method of least squares (Wilson *et al.*, 1942).  $K_s \pm 2\sigma$  was found to be  $0.0016 \pm 0.0002$ .

Analogous experiments were carried out to determine the  $K_s$  value for inductions carried out under anaerobic conditions. These experiments differed from the aerobic ones only in the fact that nitrogen rather than air was bubbled through the tubes. The results of a representative experiment are detailed in table 2. It will be noted from a comparison of tables 1 and 2 that anaerobiosis in the presence of an external energy supply does not affect materially the rate of enzyme formation. The rates attained at the various  $\alpha$ -methyl glucoside concentrations under the two conditions are comparable. The data of table 2 yield  $K_s \pm 2\sigma$  of  $0.0020 \pm 0.0002$  which is in good agreement with that found in the aerobic experiments.

*K<sub>s</sub> of  $\alpha$ -methyl glucoside as an inhibitor of maltase.* To make the needed comparison it was now necessary to determine the combining

TABLE 1

Rate constant of aerobic maltase synthesis at varying concentrations of  $\alpha$ -methyl glucoside

(S) CONCENTRATION [M] OF $\alpha$ -METHYL GLUCOSIDE	(v) RATE CONSTANT OF MALTASE SYNTHESIS	(S)/(v)
0.154	0.0190	8.10
0.052	0.0184	2.80
0.010	0.0163	0.63
0.002	0.0147	0.14
0.001	0.0136	0.08

Cells were induced aerobically in the presence of glucose (3.3 mg/ml) as energy source. Rate constants determined from the slopes of the linear portions of the semi-log plots of enzyme activity versus time.

TABLE 2

*Rate constants of anaerobic maltase synthesis at varying concentrations of  $\alpha$ -methyl glucoside*

(S) CONCENTRATION (M) OF $\alpha$ -METHYL GLUCOSIDE	(v) RATE CONSTANT OF MALTASE SYNTHESIS	(S/v)
0.154	0.0229	6.7
0.052	0.0173	2.9
0.010	0.0157	0.64
0.002	0.0132	0.15
0.001	0.0119	0.08

Cells were induced under  $N_2$  in the presence of glucose (3.3 mg/ml) as an energy source. Rate constants were determined from the slopes of the linear portions of the semi-log plots of enzyme activity versus time.

capacity of  $\alpha$ -methyl glucoside with the maltose utilization by various concentrations of  $\alpha$ -methyl glucoside at differing levels of maltose. A meaningful interpretation of such data requires that the following conditions be satisfied: (1) The maltase activity of the cells being studied must be rate limiting relative to glucose utilization. (2) Enzyme must neither be formed nor lost during the test. (3) The  $\alpha$ -methyl glucoside must not interfere significantly with the glucosylase system.

The first two conditions were met easily by employing cells which were partially induced and stabilized with ultraviolet. In cells so prepared, the maltose fermenting capacity remains at a constant level of one-half or less the rate of glucose utilization.

That the third condition is satisfied was shown by examining the effect of  $\alpha$ -methyl glucoside on the fermentation of limiting amounts of glucose. In order to make the conditions strictly comparable to the maltose utilizing inhibition experiments, half-induced, ultraviolet stabilized cells were used. The resulting cells were washed, resuspended in pH 4.5 buffer, and the rate at which they anaerobically fermented one mg of glucose in the presence and absence of  $\alpha$ -methyl glucoside was followed manometrically. Representative results are given in Figure 2. The initial concentration of glucose (0.5 mg per ml) is below saturation, hence, the fermentation rate begins to drop immediately and continues doing so as the glucose is consumed. For the purpose of plotting, the amount of glucose remaining at any given time was calcu-

lated from the  $CO_2$  evolved during the corresponding period. There is clearly no evidence that the presence of  $\alpha$ -methyl glucoside has any detectable inhibitory effects on glucose fermentation even at concentrations of glucose that are far below the saturation levels.

A determination of the  $K_m$  value of maltose as a substrate was made and found to be in the neighborhood of 0.01 M. This relatively low combining capacity made it possible to survey the effects of  $\alpha$ -methyl glucoside at lower maltose concentrations than might otherwise have been possible. In particular, it was feasible to examine the region in which maltose was not saturating its enzyme.

As noted earlier, all the experiments on inhibition of maltose fermentation with  $\alpha$ -methyl glucoside were carried out with half-induced, ultraviolet stabilized cells, and the rates were determined manometrically at 30 C under anaerobic conditions.

It became apparent early that  $\alpha$ -methyl glucoside was acting as a noncompetitive inhibitor since no concentration of maltase could achieve a complete reversal of the inhibitory effect. This property of the inhibition is exemplified in figure 3 which illustrates the inverse plot commonly employed to distinguish between the various types of inhibition (Umbreit *et al.*, 1949). It is evident that at both concentrations depicted both the slopes and the intercepts are modified

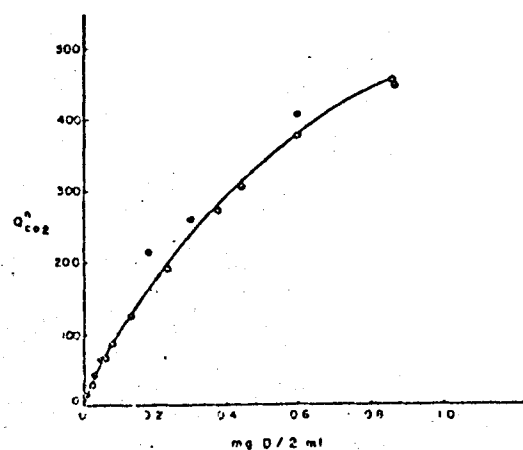


Figure 2. A test of the ability of  $\alpha$ -methyl glucoside (0.155 M) to inhibit glucose fermentation at limiting concentrations of the latter. Half shade circles correspond to fermentation in the presence of  $\alpha$ -methyl glucoside and open circles to the controls.

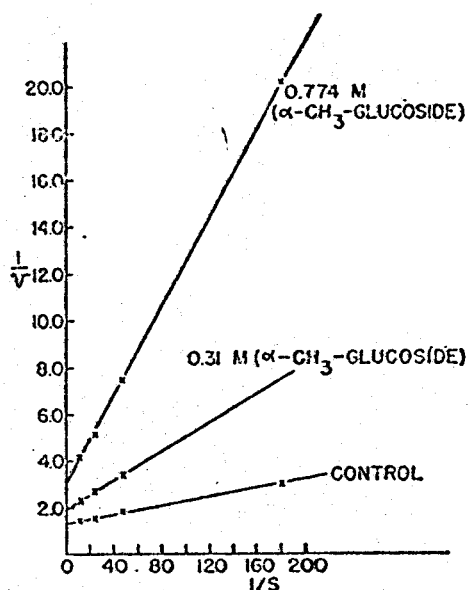


Figure 3. A double inverse plot of rate of maltose utilization against maltose concentration in the presence and absence of  $\alpha$ -methyl glucoside and illustrating noncompetitive nature of the inhibition.

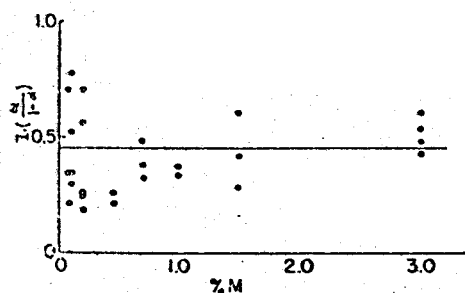


Figure 4. A Hunter-Downs (1915) plot of the data of table 3, demonstrating the noncompetitive nature of the inhibition of maltose fermentation by  $\alpha$ -methyl glucoside.

as compared with the controls. This is the type of result characteristic of noncompetitive inhibition. Another method (Hunter and Downs, 1915) of examining this question is to plot  $[I] \frac{\alpha}{1 - \alpha}$  against substrate concentration, where  $[I]$  is the concentration of the inhibitor being tested and  $\alpha$  is the fractional activity observed (i.e.,  $\frac{v_i}{v}$  where  $v_i$  is the velocity in the presence of inhibitor and  $v$ , the rate observed in its absence). In the case

of a competitive inhibitor,  $[I] \frac{\alpha}{1 - \alpha}$  is a linear function of the substrate concentration. For a noncompetitive inhibitor, on the other hand,  $[I] \frac{\alpha}{1 - \alpha}$  is independent of substrate concentration. That this is the situation in the present case can be seen from figure 4 which summarizes data obtained at seven different concentrations of maltase and three concentrations of  $\alpha$ -methyl glucoside.

As shown by Hunter and Downs (1915) the dissociation constant  $K_i$  of a noncompetitive inhibitor possessing the properties indicated in

TABLE 3

The determination of the dissociation constant ( $K_i$ ) of  $\alpha$ -methyl glucoside and the maltose utilizing enzyme

EXPT	$\alpha$ -METHYL GLUCOSIDE	MALTOSE	$\frac{V_i}{V}$	$K_i$
1	M 0.0775	% 0.067	0.90	0.70
		0.08	0.816	0.34
		0.10	0.832	0.34
		0.20	0.88	0.56
		0.50	0.77	0.26
		1.00	0.83	0.38
2	0.0775	3.00	0.888	0.61
		0.10	0.88	0.52
		0.20	0.76	0.25
		0.75	0.755	0.32
		1.50	0.786	0.29
		3.00	0.815	0.54
3	0.155	0.10	0.835	0.77
		0.20	0.626	0.26
		0.75	0.757	0.48
		1.50	0.80	0.60
		3.00	0.727	0.43
4	0.310	0.20	0.695	0.70
		0.75	0.55	0.38
		1.50	0.577	0.42
		3.00	0.615	0.48

Mean.....  $0.46 \pm 0.057^*$

\* Two standard deviations of the mean,  $2\sigma_m$ .

Anaerobic maltose fermentation was followed manometrically at 30 C. The cells were half-induced and ultraviolet stabilized.  $V_i$  is the rate in the presence of inhibitor and  $V$  is the rate in its absence.  $K_i$  was calculated using equation (8).

figure 4 can be determined from the following relation:

$$K_i = [I] \frac{\alpha}{1 - \alpha} \quad (8)$$

where the symbols have the meanings mentioned above. The results of 21 such determinations are summarized in table 3, from which it is seen that an average value of 0.46 was found for  $K_i$ .

#### DISCUSSION

The experiments described in the present paper were designed to see whether inducer combined specifically with existing enzyme in inducing the synthesis of new enzyme molecules. This was accomplished in terms of a comparison of the dissociation constants of  $\alpha$ -methyl glucoside as an inducer of maltase synthesis and as a complexant with the enzyme. An average  $K_i$  value of 0.0020 M was found for its inductive activity anaerobically. This is to be compared with 0.46 M as its dissociation constant with enzyme. Evidently  $\alpha$ -methyl glucoside has a 250-fold greater affinity for whatever structure it combines within its role as an inducer than it has for the enzyme molecule, the synthesis of which it can induce. These results are not in agreement with any simple interpretation of the often suggested mechanism of induction which requires that the inducer function as a specific complexant with enzyme as a primary and mandatory step in the induced synthesis of enzyme.

Experiments with two systems have, by now, provided interpretable data relevant to the role of the inducer in enzyme synthesis. They are  $\beta$ -galactosidase synthesis in *E. coli* (Lederberg, 1951; Monod *et al.*, 1951) and maltase formation in *S. cerevisiae*. These investigations have succeeded in violating one or more of the predictions derivable from the complexing concept. Under the circumstances it seems advisable to abandon this interpretation of the role of inducer.

#### SUMMARY

$\alpha$ -Methyl glucoside, an analogue of maltase, can induce the synthesis of maltase in *Saccharomyces cerevisiae*. Experiments were performed designed to see whether  $\alpha$ -methyl glucoside combined with maltase in the course of stimulating its synthesis. The dissociation constant of  $\alpha$ -methyl glucoside as an inducer

was measured and found to be  $0.0016 \text{ M} \pm 0.0002$  aerobically and  $0.0020 \text{ M} \pm 0.0002$  anaerobically. Measurement of the dissociation constant between  $\alpha$ -methyl glucoside and the maltase utilizing enzyme gave a value of 0.46 M. The marked discrepancy between these values indicates that  $\alpha$ -methyl glucoside does not combine with maltase in the process of stimulating the synthesis of this enzyme.

These results, along with others on the synthesis of  $\beta$ -galactosidase, suggest the necessity of abandoning the complexing concept to explain the role and specificity of inducers in enzyme synthesis.

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